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# ASSESSING A LABORATORY TEST ON FOOD PREFERENCES OF THE MELON FRUIT FLY, *Bactrocera cucurbitae* (DIPTERA: TEPHRITIDAE)

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## ABSTRACT

Controlling the melon fruit fly, *Batrocera cucurbitae* (Diptera: Tephritidae) can be achieved by employing food bait trapping techniques that could reduce reliance on insecticides. However, the success of this method depends on several factors which include the sweetness and nutritional value of the food, as well as other population-affecting factors. As a result, performing laboratory studies becomes crucial in order to observe the behaviour and tendencies of the melon fruit fly towards their dietary choices. Therefore, the objective of this study was to evaluate the food preferences of melon fruit flies in the laboratory before applying food in baited traps for field studies. The study employed a choice and no choice experiment, presenting four types of foods: Brewer's yeast, banana, cucumber, and Melon Manis Terengganu (MMT). Each experiment involved releasing a four-day-old adult female of *B. cucurbitae* into a rearing cage containing the four foods for choice and each food (individually) for no-choice experiment. The fly's foraging behaviour was observed and recorded for a duration of two hours with five replications. The results indicated significant differences *($P<0.05$)* in the duration of food consumption by the flies when exposed to different host foods for both experiments. Notably, the yeast recorded the significantly longest duration of food consumption with 19.29±3.73 minutes for choice experiment and 21.44±8.22 minutes for no choice experiment, compared with other food hosts. However, no significant differences *($P>0.05$)* were observed in the number of food visits, duration of food visits, and number of consumptions across all the food hosts. These findings highlight the importance of using yeast as one of the food baits in the management of melon fruit flies. Its incorporation enhances pest control methods, contributing to a more efficient and environmentally friendly approach to managing this pest.

**Keywords:** Food bait, food preferences, laboratory test, fruit fly, *Bactrocera cucurbitae*

## ABSTRAK

Kawalan Lalat Buah Melon, *Bactrocera cucurbitae* (Diptera: Tephritidae) boleh dicapai dengan menggunakan teknik perangkap umpan makanan untuk mengurangkan pergantungan kepada racun serangga. Walau bagaimanapun, kejayaan kaedah ini bergantung kepada beberapa faktor termasuk kemanisan dan kandungan nutrisi dalam makanan, serta faktor lain yang mempengaruhi populasi. Justeru, kajian awal di makmal adalah penting untuk memerhatikan tingkah laku dan kecenderungan lalat buah melon terhadap pilihan pemakanan mereka. Oleh itu, objektif kajian ini adalah untuk menilai pemilihan makanan lalat buah melon di makmal sebelum mengaplikasikan makanan dalam perangkap berumpan makanan untuk kajian lapangan. Kajian ini menggunakan eksperimen pilihan dan tiada pilihan, dengan mengemukakan empat jenis makanan: yis Brewer, pisang, timun, dan Melon Manis Terengganu (MMT). Setiap eksperimen melibatkan pelepasan seekor betina dewasa *B. cucurbitae* berumur empat hari ke dalam sangkar eksperimen yang mengandungi empat makanan untuk eksperimen pilihan dan sejenis makanan bagi eksperimen tanpa pilihan. Tingkah laku pencariaan makanan lalat telah diperhatikan dan direkodkan selama dua jam dengan lima ulangan. Keputusan menunjukkan perbezaan yang signifikan *(P<0.05)* dalam tempoh masa makan oleh lalat apabila terdedah kepada makanan yang berbeza untuk kedua-dua eksperimen. Terutamanya, yis merekodkan tempoh makan yang paling lama dengan ketara iaitu 19.29±3.73 minit untuk eksperimen pilihan dan 21.44±8.22 minit untuk eksperimen tanpa pilihan, berbanding dengan makanan lain. Walau bagaimanapun, tiada perbezaan yang ketara *(P>0.05)* diperhatikan dalam bilangan lawatan makanan, tempoh lawatan makanan, dan bilangan kali makan merentas semua makanan. Penemuan ini menyerlahkan kepentingan penggunaan yis sebagai salah satu umpan makanan dalam pengurusan lalat buah melon. Penggabungannya meningkatkan kaedah kawalan perosak, menyumbang kepada pendekatan yang lebih cekap dan mesra alam untuk menguruskan perosak ini.


**Katakunci:** Perangkap makanan, pemilihan makanan, ujian makmal, lalat buah, *Bactrocera cucurbitae*

## INTRODUCTION

Diptera is one of the most biologically diverse, anatomically variable, and environmentally inventive groups of organisms, which account for 10% to 15% of all known animal species (Badii 2020). Among them, the Tephritidae, commonly known as true fruit flies, stands out as one of the largest dipteran families, encompassing approximately 4,000 species distributed across 500 genera (Radonjić et al. 2019). Globally, tephritid fruit flies rank among the most economically significant crop pests, comprising a minimum of 200 pest species (Nanga et al. 2022) and affect nearly all parts of the world where the fruit is grown (Qin et al. 2015). Malaysia have at least 100 different *Bactrocera* species, with over seventy of them already identified (Sardar et al. 2023; Wee & Shelly 2013). One of the species is the melon fruit fly, *Bactrocera cucurbitae* (also known as *Zeugodacus cucurbitae*) which is widely regarded as the most damaging pest of melons and allied crops (Poonia et al. 2024).

In the absence of control measures, the extent of losses caused by *B. cucurbitae* varies between 30% to 100% depending on the climatic conditions and susceptibility of the crop variety (Nankinga et al. 2014; Sohrab et al. 2018). Without a doubt, pesticides have been utilized to control the population of melon fruit fly. However, the majority of the tender and delicate fruits are where the gravid female lays her eggs. Upon hatching, the larvae consume the pulp, demonstrating an ability to evade contact with pesticides, thereby reducing the effectiveness of the chemicals usage (Singh et al. 2017). Hence, controlling adult flies becomes the primary focus of efforts, yet the need for repetitive spraying of synthetic insecticides raises concerns about the potential presence of hazardous residues in harvested produce (Riyaz et al. 2021). The extensive application of pesticides can also lead to significant consequences, such as environmental pollution in the form of air, water, and soil contamination, posing considerable health risks to living organisms (Sharma et al. 2019).

Therefore, there has been a transition in plant protection strategies from relying on chemicals to adopting Integrated Pest Management (IPM) approaches such as biological, mechanical and mass-trapping control (Bade et al. 2022). In Malaysia, bait trapping control using methyl eugenol (ME) and cue lure (CL) has been frequently utilized, and extensive research has been conducted in this area to manage the country's fruit fly population. Wee & Shelly (2013) stated that during a 12-week sampling period on a fruit farm in Selangor, traps baited with solid wafer lures containing ME and CL captured significantly fewer males than traps baited with liquid lures, contrary to findings in Hawaii. This was true for all five ME-responding taxa analyzed and one of the three CL-responding species. Another research reveals that the response pattern of male *B. cucurbitae* fruit flies to CL and zingerone (ZN) was similar, demonstrating an increasing age-dependent response to the attractions of the baits as age and sexual maturity progressed (Wee & Ooi 2022).

Aside from that, a study examining fruit fly responses to curry leaf odor found that male fruit flies were more attracted to the aroma, with an attraction rate of around 80%, compared to approximately 57.5% for female fruit flies (Widihastuty et al. 2023). Meanwhile, food bait trapping techniques have been used to capture female *Batrocera* species since 1918 by using baits based on protein solutions, fermenting sugar solutions, fruit juices, and vinegar (IAEA 2018). Female flies are drawn to the scent emitted by the host or food because they need a food source to continue producing and sustaining eggs or their species for the rest of their lives (Ismail 2012; Roh et al. 2021). Food bait traps, utilizing locally available materials, have proven to be an environmentally friendly method for monitoring and controlling fruit flies in the field (Abinaya et al. 2020).

However, food bait trapping effectiveness varies depending on factors such as fruit fly diet, fruit fly species, and other factors influencing fruit fly population (Nanga et al. 2022). According to Smith et al. (2015), laboratory tests are vital for establishing a definitive basis in field trials, ensuring the measurement of outcome variables and generating high-quality data to verify the effectiveness of baits. As a result, preliminary research is necessary in order to examine the melon fruit fly's behavior and tendencies to their dietary choices. Therefore, this study was carried out in the laboratory to examine the food preferences of *B. cucurbitae* before employing food in baited traps for field studies.

## MATERIALS AND METHODS

### Food Hosts Preparation

All the food hosts were bought from local market namely the Brewer's yeast, banana, cucumber, and Melon Manis Terengganu (MMT). All the fruits were sliced with the same thickness and weight (2 mm: 3 gram) while the yeast was prepared in 5:1 ratio of yeast and water to make a yeast paste. All the food hosts were placed in petri dish layered by filter paper to absorb any excess moistures. The method was adopted and modified from (Liu et al. 2018).

**Adult of *Bactrocera cucurbitae***

The experiment and insect culture were carried out at Laboratory of Entomology, Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu. The adult *B. cucurbitae* flies used in this experiment were obtained from established colonies from the Quarantine Laboratory, Horticultural Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor and placed in a rearing cage (30 x 45 x 30 cm). The cage frame was made by a rectangle steel with mesh netting (80 microns) that small enough to keep ants and other insects out but sufficient for cross ventilation. The colonies were maintained in the laboratory (28±2ºC, 70-80% RH, 12:12h L:D) for four generations to ensure the stability and purity of the flies prior to their use in the experiment. The methods for cultivating and raising *B.cucurbitae* in the laboratory were adopted and modified from previous works by Kaur et al. (2021) and Saeed et al. (2022). To initiate the experiment, five recently emerged adults were randomly selected from the stock culture and introduced into a new rearing cage. Only female *B. cucurbitae* flies that were four-days-old were utilized in the experiment. This age was chosen as it is the optimal time for foraging food and is not influenced by mating behaviours (Mack & Zhang 2023).

**Choice Bioassay**

Four types of food which are Brewer's yeast, banana, cucumber, and Melon Manis Terengganu (MMT) were arranged in separate Petri dishes (100m x 15mm). These dishes were then positioned in a rearing cage (30 x 45 x 30 cm), maintaining a 5 cm gap between them (Figure 1). A metal cage enclosed with fine netting was employed to observe the fruit flies' behaviours towards their dietary choices and to facilitate the placement of food items. Then, one pre-starved four-day-old female *B. cucurbitae* fly was released into the cage containing the four foods and plastic cups filled with water (saturated cotton strip sponge covered with cotton pads).

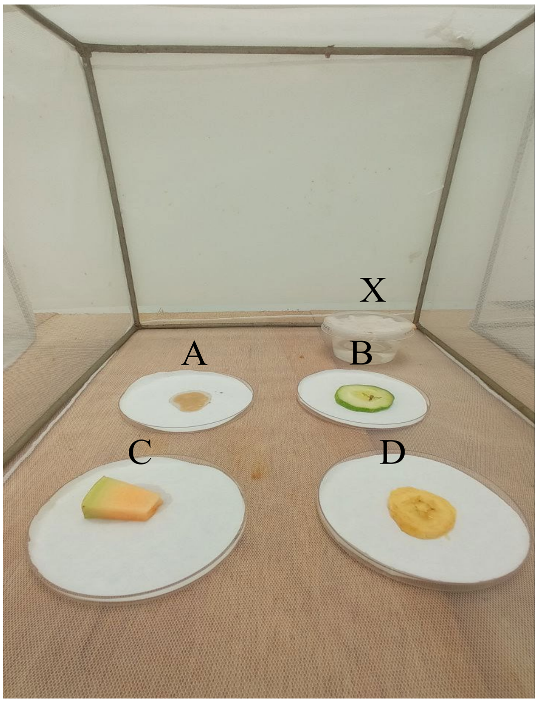


Figure 1. Experimental cage set up for choice experiment. X- water soaked-cotton; A-Brewer's yeast; B- cucumber slice; C- MMT slice; D- banana slice

The selection of female flies is based on their higher tendency to actively seek out food sources, which is essential for their continuous egg production and the sustenance of their species throughout their lifespan (Roh et al. 2021). This pre-starved fly was deprived of any food source and only given water-soaked cotton pads for 24 hours before being released into the experimental cage. This process ensures that the fly's natural inclination to find a suitable food host is more accurately assessed. The experiment was replicated five times and repeated over three generations or cycle of *B. cucurbitae* to obtain accurate result.

**No Choice Bioassay**

In a no choice experiment, four food hosts (Brewer's yeast, banana, cucumber, and MMT) were placed individually in the middle of the rearing cage with plastic cups filled with water (Figure 2). One pre-starved four-day-old female *B. cucurbitae* fly was released into the cage subjected to a 24-hour period of deprivation from any food source. The fruit flies were provided only with water-soaked cotton pads before being released into the experimental cage. The methodology for the set-up of the experiment were same as for the choice experiment. The experiment was repeated for five times and replicated over three generations of *B. cucurbitae.*

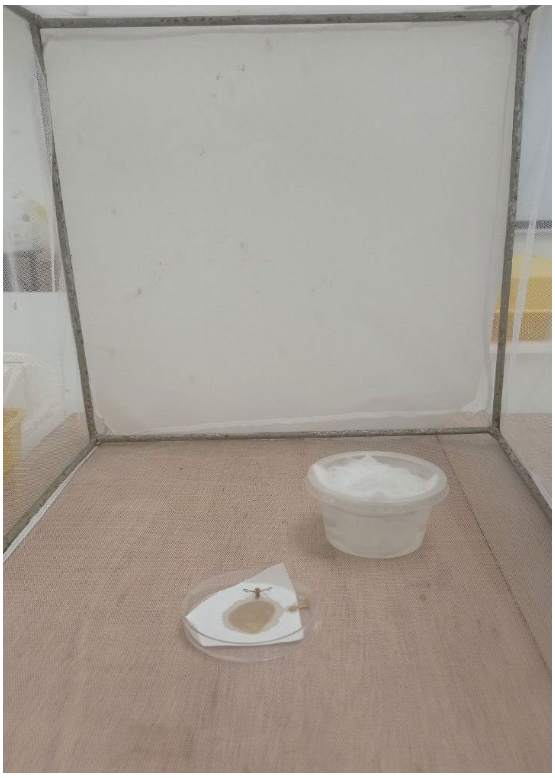

Figure 2. Experimental cage set up for no choice experiment

**Behaviours Observation**

Fruit fly behaviours were observed for 2 hours (0900-1100) with a 24-hour interval before the subsequent observation. The selected time frame was based on the peak activity of the flies, as they are most active during this period (Nor et al. 2018). The behaviour parameters recorded were; 1) number of food visit, 2) duration of visit, 3) number of consumption and 4) duration of consumption completed. The number of times the fly visit and consume food were recorded using an electronic hand counter (LINE™, Japan) while the duration of food visit and consumption process completed were recorded using a stopwatch (Diamond, China).

**Data Analysis**
The experiment design was based on a Completely Randomized Design (CRD) with five replications per treatment. Flies' behaviour (number of food visit, duration of visit, number of consumptions, duration of consumption completed) obtained from the experiment was subjected to One-way of Analysis of Variance (ANOVA) for comparison between different food hosts. Means were separated with Tukey's Range (HSD) Test at 0.05 level of significance. All data analyses were performed using Statistical Package for the Social Sciences (SPSS) software (2021

## RESULTS

**Choice Observation**
The Table 1 shows the result of observation on food preferences behaviour parameters of *B. cucurbitae* (i.e. number of food visit, duration of food visit, number of food consumption and duration of consumption completed) on four types of food hosts under choice experiment. Results show significant differences (*P<0.05*) in the duration of food consumption by the flies after exposed to different types of food hosts. Flies consume Brewer's yeast significantly longer than other food hosts with 19.29±3.73 minutes, followed by banana (11.85±1.96 minutes), MMT (7.47±1.13 minutes), and cucumber (4.73±1.02 minutes). Interestingly, when compared to other food hosts, yeast showed the lowest results in terms of the number of food visits (1.04±0.13 times), the duration of food visits (3.00±1.48 minutes), and the number of food consumptions (1.47±0.24 times).

Table 1. Food preferences behaviour parameters of *B. cucurbitae* under choice experiment

| Food Choices | Total Individuals | No. of Food Visit (Mean±SE) | Duration of Visit (min) (Mean±SE) | No. of Food Consumption (Mean±SE) | Duration of Food Consumption (min) (Mean±SE) |
|---|---|---|---|---|---|
| Brewer's Yeast | 15 | 1.04±0.13[a] | 3.00±1.48[a] | 1.47±0.24[a] | 19.29±3.73[a] |
| Banana | 15 | 1.43±0.26[a] | 3.62±1.45[a] | 2.06±0.26[a] | 11.85±1.96[ab] |
| Cucumber | 15 | 1.94±0.37[a] | 3.27±0.82[a] | 2.67±0.46[a] | 4.73±1.02[b] |
| MMT | 15 | 1.61±0.30[a] | 3.98±1.17[a] | 2.40±0.31[a] | 7.47±1.13[b] |

Means with same letters within columns were not significantly different (*P>0.05*) by Tukey's (HSD) test.

On the other hand, there were no significant differences (*P>0.05*) observed in the number of food visits, duration of food visits, and number of consumptions across all the food hosts. It was observed that female of *B. cucurbitae* preferred to visit cucumber and recorded the highest numbers (1.94±0.37 times) followed by MMT (1.61±0.30 times) and banana (1.43±0.26 times). The similar trend was also observed on a number of food consumption where the highest number of consumptions occurred was recorded on cucumber (2.67±0.46 times) compared to MMT (2.40±0.31 times) and banana (1.43±0.26 times). The duration of food visit of flies shows no significant differences (*P>0.05*) which MMT showed longer duration of visit of 3.98±1.17 minutes followed by banana (3.62±1.45 minutes), cucumber (3.27±0.82 minutes) and lastly yeast (3.00±1.48 minutes).

**No Choice Observation**
The analysis of the observation on food preferences behaviour parameters of *B. cucurbitae* (i.e. number of food visit, duration of food visit, number of food consumption and duration of

consumption completed) on four types of food hosts under no choice experiment was shown in Table 2. Overall, there was no significant difference (*P>0.05*) of the number of flies visiting food and number of times for food consumption on all types of food hosts. Banana recorded the highest number for both food visit and food consumption with 2.00±0.32 times and 3.15±0.64 times respectively, followed by MMT, cucumber and Brewer's yeast.

Table 2. Food preferences behaviour parameters of *B. cucurbitae* under no choice experiment

| Food Choices | Total individuals | No. of Food Visit (Mean±SE) | Duration of Visit (min) (Mean±SE) | No. of Food Consumption (Mean±SE) | Duration of Food Consumption (min) (Mean±SE) |
|---|---|---|---|---|---|
| Brewer's Yeast | 15 | 1.15±0.31[a] | 0.41±0.06[b] | 1.20±0.29[a] | 21.44±8.22[a] |
| Banana | 15 | 2.00±0.32[a] | 0.72±0.24[ab] | 3.15±0.64[a] | 5.90±2.15[ab] |
| Cucumber | 15 | 1.25±0.36[a] | 1.72±0.46[a] | 1.80±0.66[a] | 3.10±0.89[b] |
| MMT | 15 | 1.95±0.22[a] | 1.49±0.29[ab] | 2.70±0.24[a] | 6.27±1.79[ab] |

Means with same letters within columns were not significantly different (*P>0.05*) by Tukey's (HSD) test.

Meanwhile, the duration of visit of female flies was significantly higher (*P<0.05*) on cucumber (1.72±0.46 minutes) compared to MMT (1.49±0.29 minutes), banana (0.72±024 minutes) and the shortest time of visit is on yeast (0.41±0.06 minutes). Following the same trend in the choice experiment for duration of food consumption, it was noted that a significant difference (*P<0.05*) occurred during the period when flies consume food after being exposed to various types of hosts. Generally, flies consumed Brewer's yeast showed longer time than other food hosts with 21.44 8.22 minutes, followed by MMT (6.27±1.79 minutes), banana (5.90±2.15 minutes), and cucumber (3.10±0.89 minutes). Yeast also showed the lowest results in terms of the number of food visits (1.04±0.13 times), the duration of food visits (3.00±1.48 minutes), and the number of food consumptions (1.47±0.24 times) compared to other food hosts.

## DISCUSSION

Based on the data presented in Table 1 and Table 2 for choice and no choice experiment, it was observed that Brewer's yeast had the longest consumption period compared to other food sources. This finding suggests that *B. cucurbitae* in this study preferentially selects Brewer's yeast as a food source to support their lifecycle similar with the two preceding studies (Leblanc et al. 2010; Sruthi et al. 2022) consistently demonstrated that the yeast solution is significantly more appealing to *Bactrocera* species.

Leblanc et al. (2010) discovered that, in the case of both the oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the melon fly, *Batrocera cucurbitae* (Coquillett), significantly higher numbers of individuals from both sexes were captured in yeast-baited traps compared to those baited with synthetic food packets. More recently, Sruthi et al. (2022) also mentioned that, in the bitter gourd field, protein-baited traps demonstrated superiority with an average capture rate of 72.50 fruit flies per trap over a 9-week period. Notably, the prevalence was higher among female fruit flies, reaching 51.75 captures per trap per week.

This observation is in line with the research from Bade et al. (2022) in the previous year, which emphasizes the importance of a protein-rich diet, along with carbohydrates and sufficient moisture, for female flies to achieve sexual maturity, particularly during the preoviposition period. Moreover, tephritid fruit flies have the ability to lay eggs throughout their entire adult lifespan, given that they have access to protein-rich food (Roh et al. 2021). Consequently, females persistently seek out protein sources to provide the necessary resources for ovarian development and vitellogenesis (Wang et al. 2018).

Recent study from Gupta & Regmi (2022), stated that the yeast-based lures effectively attracted female fruit flies, owing to the presence of 13 volatile compounds such as 4-ethylphenol and 4-ethylguiacol identified from yeast conversion of p-coumaric (Wright 2015), which aligns with the fruit flies' preferences. The high protein content of Brewer's yeast, approximately 45–60%, as well as its recognized safety (GRAS) status (Jaeger et al. 2020), further supports its suitability as a preferred food choice for these flies.

Then, banana and MMT comes second for the duration of consumption in choice and no choice experiment respectively. Flies attract to both fruits compared to cucumber probably because of the higher sugar content, since fermenting sugars attract fruit flies (Bharathi et al. 2004). The sweetness level of mature MMT is around 13–19% Brix (Fauzie Jusoh et al. 2022) while banana records 18-22.8% Brix (Liew & Lau 2012). Cucumber contains lower sweetness level with only 2.2%–5.4% Brix (Kleinhenz & Bumgarner 2015). Study by Dar et al. (2020) revealed that dipteran flies, such as fruit flies, exhibit a significantly strong response to fermented sugars in the total catch of insects. Fruit flies identify sugars as a viable food source but avoid bitter-tasting substances, which are commonly toxic to both insects and mammals (Ebbs & Amrein 2007). Moreover, sugars serve as the primary energy source for fruit flies, supporting essential activities necessary for their survival and basic life functions (Wang et al. 2023).

Overall, there are no significant differences in flies' behaviour to times of food visit, duration of the visit and times of food consumption because flies spend the time only to screen the food before consuming it. Flies consume quantities of food too low to be measured and feed by extending their proboscis into the food medium (Wong et al. 2009). Typically, female *Bactrocera* flies engage in host fruit screening by using their antennae and mouthparts to touch the surface of the fruit (Nor et al. 2018). Similar to many other families of phytophagous insects, tephritid fruit flies use this behaviour as a guidance of adults to locate essential resources which may involve both chemical and visual stimuli from plants (Drew et al. 2003).

However, there is a variation in the duration of fruit fly visits to host foods between the choice and no-choice experiments. In the choice experiment, *B. cucurbitae* records no significant value, whereas it shows a significant value in the no-choice experiment with cucumber as the highest number of visits. Similar to humans, fruit flies also exhibit responses to a broad spectrum of taste chemicals and can distinguish between various taste categories, including sweet, bitter, sour, umami, and salty (Kaushik & Kain 2020). The taste system of a fly is distributed across its entire body, with the proboscis or labial palps acting as the primary taste organ, and taste sensilla present on the labellum, legs, wings, and female genitalia (Scott 2018). In this study, the observed behaviour of flies spending more time screening food in the no-choice experiment suggests that this prolonged duration may be attributed to the limited availability of a single option. This happens because flies are initially drawn to the area due to the odour, but despite a lack of interest, they exhibit a recurrent return, likely driven by the persistent smell (Matheson et al. 2022) in no choice cage. Besides, flies possess taste cells with the Gr5a receptor, essential for acceptance behaviour, while cells with the Gr66a receptor are crucial for avoidance responses (Marella et al. 2006). Conversely, they spend less time in the choice experiment, as they have additional food options.

Meanwhile, the lower result of yeast concerning the three behaviour parameters could be due to the flies spending less time probing or detecting the food. This behaviour could be explained by the fact that they find yeast to be favourable and, consequently, consume it more rapidly than other food sources. Recently, Weaver et al. (2023) also declared and identified that fruit flies, can eat for pleasure as well as necessity with greater feeding time indicating hedonic feeding drive. Additionally, several studies have found that insects individuals can feed selectively when given the opportunity, and balance the intake of different nutrients according to their preferences (Aluja et al. 2016).

## CONCLUSIONS

To conclude, Brewer's yeast demonstrated the highest attractiveness and consumption rate for *B. cucurbitae on* both choice and no choice experiment compared to other food hosts. These results showed the importance of using yeast as a food bait to effectively control melon fruit flies. Employing yeast in food bait traps can significantly enhance their efficiency after application in field research and pest management. Additional research may be necessary to validate the efficacy of using yeast for controlling melon fruit fly in the field. Furthermore, exploring the factors that attract fruit flies to yeast could contribute to the development of enhanced pest management strategies.

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## AUTHORS DECLARATIONS

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**Conflict of Interest**
All authors declare that they have no conflicts of interest to influence the findings reported in this paper.

**Ethics Declarations**
Ethics declarations are not applicable for this research.

**Data Availability Statement**
This manuscript has no associated data.

**Authors' Contributions**
Nor Aminah Azizol Azeli (NAAA) was the principal researcher, collected and identified the specimens, and discussed the findings, and wrote the first draft of the manuscript. Nur Syahida Wajdah Saidi (NSWS), Norhayati Ngah (NN) and Nur Athiqah Md Yusof (NAMY) provided materials and references, Salmah Mohamed (SM) and Nur Azura Adam (NAA) revised and refined the final draft.

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